Etiology and Serological Diagnosis Of Swine Atrophic Rhinitis

By TAKESHI SHIMIZU

1st Bacteria Section, 1st Research Division,
National Institute of Animal Health

It is urgent to ascertain the etiological agent of the swine atrophic rhinitis (AR) for the diagnosis and treatment of this disease. Therefore, many etiological theories have been offered up to the present day, and after all, it has been elucidated that AR is a transmissible disease of the swine.[4]

Since 1963, Switzer and his co-workers[1),2),7),8)] have presented the agency theory of *Alcaligenes* (*Bordetella*) *bronchisepticus* in America, but it seems that other foreign researchers have not confirmed it thoroughly yet.

In these infectious experiments, the workers have made efforts to use the possible disease-free hard, but it seems that there has been no explorative paper concerning individual etiological agent of AR used hysterectomy produced colostrum deprived (HPCD) pigs. Therefore, Shimizu et al.[9)] undertook to see if *Alc. bronchisepticus* is the causative agent of AR for HPCD pigs.

After inoculation of this bacterium, all 6 HPCD pigs revealed typical turbinate atrophy macroscopically and microscopically. This fact certifies that *Alc. bronchisepticus* is one of the important etiological agents of the swine atrophic rhinitis.

It seems that there has been no established serological diagnosis of AR up to date. In 1969, Nakagawa et al.[6)] evaluated diagnostic technique of the tube agglutination test for broncho-pneumonia caused by *Alc. bronchisepticus* in guinea pigs. Kang et al. (1970)[3)] applied that technique to the diagnosis of AR with minor change. Shimizu et al. (1970[9)]) modified this technique still more. And so, the production of agglutinin at an early stage after infection was certified.

Materials and methods

Experimental design: In the experiment undertaken by Shimizu et al.,[9)] one litter, 8 HPCD piglets from crossbred pig were used. These were divided into 3 groups, 3, 3 and 2 piglets. The first group, 3 piglets were inoculated at 2, 8, 11, 15 days of age respectively, and the second group, 3 piglets were inoculated at 8, 11, 15 days of age respectively, and the third group, 2 piglets served as non-inoculated control. The first group and the other two groups were sacrificed at 37 days, 3 months, of age respectively. Then, bacteriological, serological and pathological examinations were conducted.

Housing and feeding: All piglets were housed in individual isolation units in the breeding room of primary specific pathogen free (SPF) pigs until 37 days of age, and thereafter, the second and the third groups were housed in two separated experimental pig rooms respectively.

During the first 37 days all piglets were fed the sterilized milk formula for SPF pigs prepared by Amino Feeding Co. Ltd., and thereafter, they were fed the piglet feed which was specially prepared by the laboratory of the Nippon Formula Feed Manufacturing Co. Ltd. containing no antibiotics, arsenicals or other

drug additives.

Strain inoculated: The strain of *Alc. bronchisepticus* used in this experiment was isolated freshly from the turbinate of a pig submitted from a herd with an acute rhinitis problem.

Inoculation: The culture incubated at 37°C for 24 hours on 5 per cent sheep blood agar was suspended in the broth at the rate of 1 mg per ml, and 0.5 ml of this suspension was instilled into each nasal cavity of every piglet.

Alc. bronchisepticus recovery: Samples were obtained by cotton swabbling for the mucous membrane of the respiratory organs. They were cultured on MacConkey's medium plus 1 per cent dextrose for 48 hours at 37°C. The number of colonies was manifested shortly by the signs of − to +++. These express as follows; 0:−, 1~9:+, 10~49:++, more than 50:+++, respectively.

Pathological examination: Macroscopical observation on the turbinate atrophy adopted the Maeda et al. expression[5] which has used the signs of − to +++, according to the degree of lesions. The materials for histological investigation were collected from the whole body and fixed in a 10 per cent formalin solution following macro-ventral turbinate slices cut transversely at the first premolar teeth and decarcified with 5 per cent tricrol acid solution for 48 to 72 hours after fixation.

Antigen for serological test: Phase I colony of the strain inoculated was selected and used for antigen preparation. The culture incubated at 37°C for 24 hours on blood agar was then re-inoculated into trypticase soy broth (BBL) at the rate of 4 loopful doses per 10 ml, and thereafter, was incubated at 37°C overnight. The concentration of the antigen was adjusted to McFarland's nephelometer No. 5 with the same broth.

Agglutination test: The tube agglutination test using modified Kang et al. method[3] was adopted. Pig sera to be tested were diluted 5 times with saline, and inactivated at 56°C for 30 minutes. After two-fold dilutions were made on the inactivated sera using the antigen suspension mentioned above, resulted serum-antigen mixtures were left at 56°C for 2 hours, and then, at 4°C overnight. Thereafter, the mixtures were left at room temperature for 2 hours, and then were observed.

Standardized agglutination degree: The degree of agglutination was manifested correspondingly to the clearness of the verge, being equal to the compactness of the agglutinated sediment. The signs of − to +++ express as follows; no agglutinated sediment: −, insufficient clear: +, ordinary clear: ++, and very clear: +++. The degree of +++ and ++ are assigned positive. These are shown in Fig. 1.

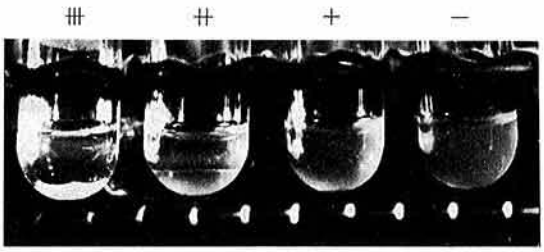

Fig. 1. Standardized agglutination degree

Results

All 6 inoculated pigs had turbinate atrophy, but 2 non-inoculated control pigs had none of it at necropsy. Of these 6 pigs damaged, 4 were severe, 1 was moderate and 1 was slight. These are shown in Table 1 and Fig. 2.

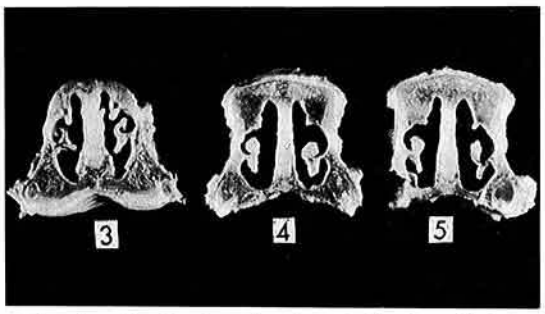

Fig. 2. Turbinate atrophy of the inoculated HPCD Pigs No. 3 to No. 5 autopsied at 37 days of age.

Histological significant changes were found in the turbinates of 6 cases inoculated. Rare-

Table 1. Turbinate atrophy and recovery of Alc. bronchisepticus in the HPCD pigs

Item			HPCD pigs							
			Inoculated						Control	
			3 (37 days)*	4 (37 days)*	5 (37 days)*	6 (3 months)*	7 (3 months)*	8 (3 months)*	1 (3 months)*	2 (3 months)*
Turbinate atrophy	Macroscopic		###	###	###	###	+	##	−	−
	Microscopic	Rarefaction of osseous core	##	##	##	###	+	###	−	−
		Inflammation of nasal mucosa	##	##	##	+	+	+	−	−
Recovery of Alc. bronchisepticus	Turbinate		###	###	###	−	−	−	−	−
	Ethmoid		###	###	###	##	##	−	−	−
	Bronchia		##	##	##	−	−	−	−	−
	Lung		+	+	+	−	−	−	−	−

* Age autopsied at

faction of osseous core was firstly noticed in 3 cases sacrificed at 37 days of age. Furthermore, these bone tissues already disappeared and were replaced by cartilage in 2 cases of 3 sacrificed at 3 months of age. Some of these 6 cases showed osteoclastic changes accompanying osteoclasts around the thin scattered bone trabeculae, although bone formation can be found at the periosteum. Besides the osseous lesion, inflammatory changes were seen in the nasal mucosa of all 6 cases. On the contrary, there was no change in 2 control cases.

Alc. bronchisepticus was recovered at necropsy in large numbers from the turbinate, ethmoid, in moderate numbers from the bronchia, in a few numbers from the lung in all 3 piglets 37 days of age, but recovered only from the ethmoid of the 1 of 3 pigs 3 months of age (See Table 1).

The degree and incidence of sneezing was noticeable since 20 days post-inoculation in all these pigs inoculated. Sneezing was never violent in these pigs though severe turbinate atrophy appeared in the 3 pigs of the first group. Any other marked symptom was not observed in all the pigs.

Agglutinin titers for the antigen in those pigs inoculated were 1:10 to 1:20 at 37 days old necropsy, and 1:40 to 1:80 at 3 months old necropsy while these titers were increasing week in, week out. But the titers in the non-inoculated control pigs were never seen all the time. These are shown in Fig. 3.

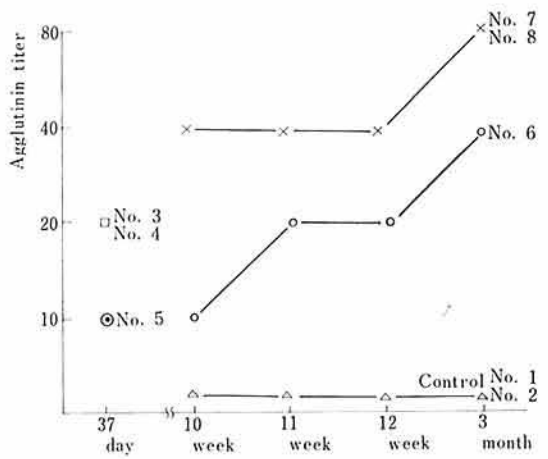

Fig. 3. Increase of agglutinin titer in the HPCD pigs.

Discussion

Experimentally induced *Alc. bronchisepticus* rhinitis resulted in macroscopic turbinate atrophy in 100 per cent of the inoculated pigs. Microscopic pathological examination of these turbinates revealed also rarefaction of the osseous core and inflammatory change in the

nasal mucosa. These pathological changes are similar to the description by Duncan et al.[1] even if there are a few differences in detail.

Inoculated organism was recovered from all 3 piglets, that is, from every turbinate ethmoid, trachea, lung at 37 days old necropsy, and recovered from only 1 of the 3 pigs, that is, from only ethmoid at 3 months old necropsy. But even in the later case, the gross lesion was revealed in all these 3 pigs. These tendencies are in line with Ross et al. report.[7]

Therefore, the tendency is confirmed, that is, inoculated, multiplied and damage-produced organism in the pig can decrease and even disappear in the respiratory tract of the pig after 3 months of inoculation.

Using the technique modified by Shimizu et al.[9] as shown above, the agglutinin was noticed as early as 37 days old, and the transition of the titer was enlightened. This technique had been certified concerning its specificity by the result shown in Fig. 3.

References

1) Duncan, J. R., et al.: Pathology of experimental *Bordetella bronchiseptica* infection in swine. Atrophic rhinitis. *Am. J. Vet. Res.* **27**, 457–466 (1966).
2) Harris, D. L. and Switzer, W. P.: Turbinate atrophy in young pigs exposed to *Bordetela bronchiseptica*, *Pasteurella multocida* and combined inoculum. *Am. J. Vet. Res.* **29**, 777–785 (1968).
3) Kang, B. K., Koshimizu, K. and Ogata, M.: Studies on the etiology of infectious atrophic rhinitis of swine. II. Agglutination test on *Bordetella bronchiseptica* infection. *Jap. J. Vet. Sci.* **32**, 295–306 (1970).
4) Kemeny, L. J., Littledike, E. T. and Cheville, N. F.: Experimental transmission of atrophic rhinitis in pigs. Effect of diet. *Cornell Vet.* **50**, 502–517 (1970).
5) Maeda, M., Inui, S. and Konno, S.: Lesions of nasal turbinates in swine atrophic rhinitis. *Nat. Inst. Anim. Hith Quart.* **9**, 193–202 (1969).
6) Nakagawa, M. et al.: Some observations on diagnosis of *Bordetella bronchiseptica* infection in guinea pigs. *Exp. Animals*, **18**, 105–116 (1969).
7) Ross, R. F., Duncan, J. R. and Switer, W. P.: Turbinate atrophy in swine produced by pure cultures of *Bordetella bronchiseptica*. *Vet. Med.* **58**, 566–570 (1963).
8) Ross, R. F., Switzer, W. P. and Duncan, J. R.: Comparison of pathogenicity of various isolates of *Bordetella bronchiseptica* in young pigs. *Canad. J. Comp. Med. Vet. Sci.*, **31**, 53–57 (1967).
9) Shimizu, T., Nakagawa, M., Shibata, S. and Suzuki, K.: Atrophic rhinitis produced by intranasal inoculation of *Bordetella bronchiseptica* in hysterectomy produced colostrum deprived pigs. *Cornell Vet.* [In press.]